WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 7, 545-555. Research Article ISSN 2277 – 7105

VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF MELOXICAM AND AMOXICILLIN SODIUM IN BULK AND COMBINED DOSAGE FORM

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Article Received on
06 July 2014,

Revised on 30 July 2014,
Accepted on 25 August 2014

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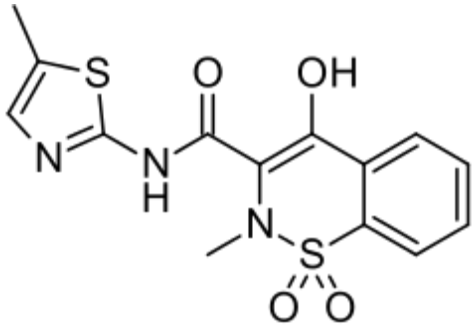
ABSTRACT

A new simple, precise, accurate and selective RP-HPLC method has been developed and validated for simultaneous estimation of Meloxicam (MEL) and Amoxicillin sodium (AMXS) in injectable dosage form. The method was carried out on a C_8 (250 mm × 4.6 mm i.d., 5µm) column with a mobile phase consisting of Phosphate Buffer, Acetonitrile and Methanol in the ratio of (45:10:45v/v/v) and flow rate of 1.5 ml/ min. The detection was carried out at 233nm.The retention time for MEL and AMXS were found to be 7.548 and 4.837 min respectively. The MEL and AMXS followed linearity in concentration range of 1-7µg/mL and 20-150µg/mL respectively with r^2=0.9990.The amount of both drugs estimated by the proposed method was found to be in good agreement with labelled claim. The developed method was validated for precision, accuracy, sensitivity, robustness and ruggedness. The developed method can be used for routine analysis of titled drugs in combined dosage form.

KEYWORDS: Meloxicam, Amoxicillin sodium; RP-HPLC, Validation, C-8 column.

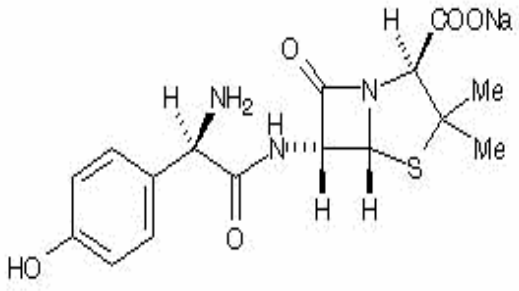
INTRODUCTION

Meloxicam (MEL), IUPAC name 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl-2H-1, 2 benzothiazine-3-carboxamine-1, 1-dioxide, is an NSAID useful for treating acute respiratory disease in veterinary [1]. It is soluble in dimethyl sulfoxide [2], slightly soluble in methanol.



The drug is official in Indian pharmacopeia and British pharmacopoeia and estimated by liquid chromatographic methods [3, 4], it was approved by US-FDA in 2000[5]. Literature survey revealed RP-HPLC method for estimation of MEL in biological samples [6] ,RP-HPLC method for the determination of MEL in combination with other oxicam drugs[7] .Potentiometric and HPLC methods for the determination of Meloxicam in Bulk and drug formulations[8].Spectrophotometric simultaneous determination of Meloxicam and Amoxicillin sodium in combined dosage form [9] , UV-Spectrophotometric methods have been studied for the determination of MEL in bulk and pharmaceutical formulations [10,11] .

Amoxicillin sodium (AMXS), IUPAC name sodium (2S, 5R, 6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl) acetyl] amino]-3, 3-dimethyl-7-oxo-4-thia-1-azabicycloheptane-2-carboxylate, is an antibiotic [12]. It is freely soluble in water; sparingly soluble in ethanol [13].The drug is official in Indian pharmacopeia and British pharmacopoeia and estimated by liquid chromatography [14, 15]. It was approved by UF-FDA in 1999[16].



MATERIALS AND METHODS

Chemicals

Meloxicam was the gift sample from Nirmala College of Pharmacy and Amoxicillin sodium was purchased from Indian market manufactured by Ranbaxy, Hyderabad. Potassium dihydrogen phosphate, HPLC grade Acetonitrile, Methanol, O-phosphoric acid were purchased from Merck (Mumbai) and HPLC grade water from cystron laboratories.

Instrumentation

Analysis was performed on waters HPLC 717 plus equipped with UV detector, Auto sampler and Inertsil C_8 column compartment with Empower 2 software. Other equipment used in the study was analytical balance (DENVER) and P^H meter (EUTECH instrument). Ultra sonic bath (UNICROME ASSOCIATES: UCA-701).

Chromatographic conditions

Inertsil C_8 column (250 mm × 4.6 mm i.d., 5µm) was used for chromatographic separation. The mobile phase composed of Buffer, Acetonitrile, and Methanol in the ratio of (45:10:45v/v/v); at a flow rate of 1.5ml/min with run time 10 min. Mobile phase and sample solutions were filtered through a 0.45 µm membrane filter and degassed. The detection of both drugs was carried out at 233 nm.

Method Development

Standard stock solutions of 10mg/ml of Meloxicam and 200mg/ml of Amoxicillin sodium were prepared separately using diluent (Acetonitrile: water-70:30v/v). The MEL stock solution was diluted with diluent to give working standard solution containing 1-7µg/ml concentration. Similarly the Amoxicillin sodium stock solution was diluted with diluent to give working standard solution in the range 20-150 µg/ml. These solutions were filled into vials and placed in vial holder. The linearity was determined separately for MEL and AMXS by injecting eight concentrations of both drugs prepared in diluent and calibration curves were constructed by plotting area against the respective concentrations.

Validation of Method

The HPLC method was validated in accordance with ICH guidelines. The system precision of the method was verified by six replicate injections of standard solution containing Meloxicam and Amoxicillin sodium. The method precision was carried out for the analyte six times using the proposed method. Repeatability was measured by multiple injections of homogenous sample of MEL and AMXS. Accuracy was carried out by percentage recovery studies at three different concentration levels. To the pre-analysed samples solution of MEL and AMXS, a known amount of standard drug powder of MEL and AMXS were added at 50, 100, 150% level. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is a procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. Sensitivity of the proposed method was estimated in terms

of limit of detection (LOD) and limit of quantification (LOQ) and was determined using the formulae; LOD =3.3 × ASD/S and LOQ = 10 × ASD/S, where, ASD is the average standard deviation and S is the slope of the line. Robustness was evaluated by making deliberate variations such as variation of wavelength, flowrate and change in mobile phase composition. The robustness of the method was studied for MEL and AMXS. Ruggedness of the method was performed by two different analysts using same experimental and environmental conditions. It was performed by injecting 1µg/ml of MEL and 20 µg/ml solutions of AMXS, respectively. The system suitability parameters such as resolution, number of theoretical plates and tailing factor were studied. Stability of sample solution was established by the storage of sample solution at 25^0c for 12hr and 24hrs. Sample solution was reanalysed after 12 hrs and 24 hrs time intervals and assay was determined for MEL and AMXS and compared against fresh sample.

Analysis of Formulation

To determine the content of MEL and AMXS in injection formulation (MEL 5mg, AMXS 100mg) an accurately weighed drug powder equivalent to 10 mg of MEL and 200mg of AMXS were transferred into 200mL volumetric flask, dissolved in 150mL of diluent and sonicated for 5 min. After achieving complete solubility of the drug, the volume was made up to the mark using diluent .The solution was filtered through the 0.45 µm nylon syringe filter. From the filtrate a 1mL solution was transferred into 50 mL volumetric flask and volume was made up to the mark with diluent to obtain a concentration of 1µg/mL of MEL and 20µg/mL of AMXS which was then subjected to proposed method and the amounts of MEL and AMXS were determined using calibration curves.

RESULTS

The proposed chromatographic system was found suitable for effective separation and quantitation of MEL (RT 7.548min) and AMXS (RT 4.837min) with good resolution, peak shapes and minimal tailing. The overlay UV spectra and typical chromatogram were shown in Figures 1 and 2.

The individual chromatograms for AMXS- API and MEL -API were shown in Figure 3 and4. Both the drugs were found to give linear detector response in the concentration range under study with correlation coefficient of 0.9990 for both MEL and AMXS. The MEL and AMXS have followed linearity in the concentration range of 1-7µg/mL and 20-150µg/mL respectively Figure 5. Percent recoveries for MEL and AMXS were 99.9-100.8 and 99.4-

101.2%. %RSD for injectable dosage form analysis, recovery studies and intra and inter-day precision studies was less than 2. LOD and LOQ were found to be 0.034µg/mL and 0.105µg/mL for MEL and 1.034µg/mL and 3.134µg/mL for AMXS.

The method precision and inter-day precision were evaluated on the basis of % RSD value and found to be in the range 0.992-1.065 and 1.567-1.356%. As the RSD values were < 2%, the developed method was found to be precise (Table 1). The accuracy of the method studied at three different concentration levels i.e. 50, 100, 150% showed acceptable recoveries in the range of 99.9-100.8%for MEL and 99.4-101.2% for AMXS (Table2).
The LOD for MEL and AMXS was found to be 0.034 and 1.034 µg/mL respectively. Further the LOQ for MEL and AMXS was found to be 0.105 and 3.134 µg/mL respectively. Robustness of the method was studied by making deliberate changes in the chromatographic conditions like flow rate (± 0.2 mL/min), wave length (± 5nm) and mobile phase composition (± 2%). The validation parameters were summarized in (Table 3).

The results of robustness study of the developed method was validated by change in flow rate , change in wave length and change in mobile phase ratio and the % RSD of those variations are less than 2 (Table 4).

When the method was performed by two different analysts under the same experimental and environmental conditions it was found to be rugged and % RSD (<2%) indicating ruggedness of the method. The system suitability parameters such as number of theoretical plates and tailing factor were studied and shown in (Table 3).

Stability of sample solution was established by the storage of sample solution at 25^0c for 12hr and sample was reanalysed after 24 hr and assay was determined for the compounds (MEL and AMXS) and compared against fresh sample. Sample solution did not show any appreciable change in assay value (% RSD<2) when stored at ambient temperature up to 24 hrs.
Six replicates of sample solutions containing 1µg/ml for MEL and 20µg/ml for AMXS were injected for quantitative analysis. The amounts of MEL and AMXS estimated were found to be 100.2 and 100.1% respectively. A good separation and resolution of both drugs indicates that there was no interference from the excipients commonly present in pharmaceutical combined dosage formulations. The results were shown in (Table 5).

DISCUSSION

The developed RP-HPLC method was found suitable for simultaneous estimation of MEL and AMXS with good resolution, peak shapes and minimal tailing. The peak areas of the drug were reproducible as indicated by low coefficient of variance indicating the repeatability of the proposed method. High correlation coefficient of 0.999 showed the stable linear detector response in different concentration ranges of both the drugs.

The proposed method was validated as per ICH guidelines. The method exhibited good selectivity and sensitivity. Percent recoveries for MEL and AMXS were 99.9-100.8 and 99.4-101.2% respectively, indicating the accuracy of the proposed method. Low LOD and LOQ values indicate high sensitivity of the proposed method. The %RSD values of less than 2 for intra and inter day variation studies indicated that the proposed was precise. The developed method was studied for percentage recovery at three concentration levels and %RSD values of less than 2 were found which were in acceptable limits indicates the method was accurate. Low %RSD values of less than 2 in variation of flow rate, wave length and mobile phase ratio indicates the method was robust. When the method was performed by two different analysts under the same experimental and environmental conditions and %RSD was found to be less than 2 indicating the ruggedness of the proposed method. The results from solution stability experiments confirmed that sample was stable up to 24 hr. during assay determination. The sample recoveries of MEL and AMXS from the commercial injectable dosage form were in good agreement with respective label claim indicating that there were no interferences from the commonly used tablet excipients and buffer used in analysis.

Table 1: Precision of Developed Method

S.No	Method precision				Inter-day precision			
	MEL		AMXS		MEL		AMXS	
	RT	Area	RT	Area	RT	Area	RT	Area
1	7.30	2768382	4.73	1955771	6.88	1636888	4.54	1149330
2	7.27	2733227	4.71	1954297	6.91	1686470	4.56	1136863
3	6.90	2790457	4.56	1970263	7.06	1664712	4.71	1165579
4	6.82	2729957	4.51	1959561	7.13	1655283	4.78	1140962
5	6.74	2779736	4.47	1989629	7.08	1653179	4.53	1142306
6	6.73	2790549	4.47	1995135	7.00	1642573	4.59	1176137
Mean	6.96	2765385	4.57	1970776	7.01	1656517	4.61	1151863
±SD		27444.654		30888.39		17645.044		15613.71
% RSD		0.992		1.567		1.065		1.356

Table 2: Accuracy Data

% Level of recovery	Area	Amount of sample added (µg/ml)	Amount of API added (µg/ml)	Amount found (µg/ml±SD)	Recovery %±SD	%RSD
			MEL			
50%	1453997	10.2	5.51	15.7	100.7	
	1350938	10.2	5.10	15.3	100.8	
	1349007	10.2	5.10	15.3	100.8	0.030
100%	2776237	10.2	10.5	20.7	100.7	
	2786326	10.2	10.6	20.8	100.1	
	2774362	10.2	10.5	20.7	100.7	0.330
150%	4209345	10.2	15.9	26.1	100.8	
	4255613	10.2	16.2	26.4	100.1	
	4380328	10.2	16.7	26.9	99.9	0.500
			AMX			
50%	987644	200	100.2	300.2	99.8	
	970167	200	100.8	300.8	99.4	
	969819	200	100.2	300.2	100.0	0.290
100%	1972088	200	200.1	400.1	100.8	
	1977063	200	200.1	400.1	101.0	
	1957542	200	200.3	400.3	100.9	0.130
150%	2995626	200	300.1	500.1	100.4	
	3020650	200	300.8	500.8	100.2	
	3108068	200	300.2	500.2	101.2	0.530

TABLE 3: VALIDATION AND SYSTEM SUITABILITY PARAMETERS

Parameter	**MEL**	**AMXS**
Range (µg/ml)	1-7	20-150
Slope	53235	19622
Intercept	53235x+94528.5	19622x+98452.1
Correlation coefficient (R^2)	0.999	0.999
Retention time	7.548±0.5	4.837±0.2
Precision (intra and inter day)% RSD	<2	<2
Accuracy	99.9-100.8	99.4-100.9
LOD(µg/ml)	0.034	1.034
LOQ(µg/ml)	0.105	3.134
Tailing factor	1.15	1.25
Theoretical plates	5225	4914
Resolution	7.63	

Table 4: Influence of Flow Rate, Wavelength And Mobile Phase Composition on Analytical Parameters

Parameter		MEL			AMXS	
	RT	Area	Tailing	RT	Area	Tailing
Flowrate(±0.2ml/min)						
1.3	8.56	3912203	1.14	5.45	2793393	1.27
1.5	7.54	2768382	1.16	4.83	1955771	1.27
1.7	6.44	3050896	1.14	4.14	2172800	1.25
Wavelength(±5nm)						
228	7.98	2657389	1.06	5.13	2285608	1.05
223	7.54	2768382	1.16	4.83	1955771	1.27
238	7.98	3549167	1.05	5.13	1958862	1.05
Mobile phase composition(±5%v/v)						
45:40:15	6.86	4967800	1.10	5.45	3544920	1.25
45:45:10	7.54	2768382	1.16	4.83	1955771	1.27
45:50:05	9.34	6631136	1.12	5.95	4743865	1.30

Table 5: Assay of Commercial Formulation

Drug	Label claim(mg/tablet)		Calculated value (ml±SD/tablet)	% of Assay
MEL	10		10.2	100.2
AMXS	200		200.20	100.1

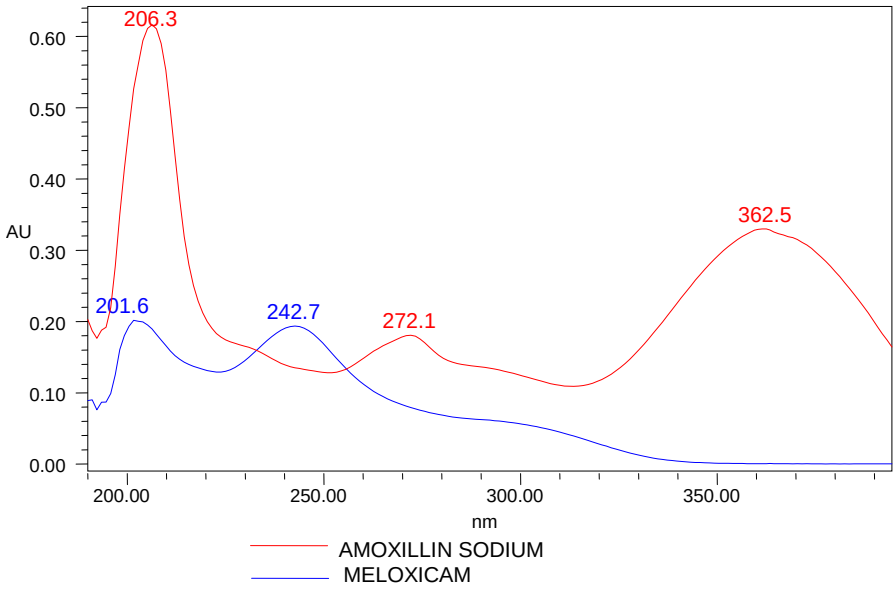


Figure 1: Overlay UV Spectra of Standard MEL and AMXS

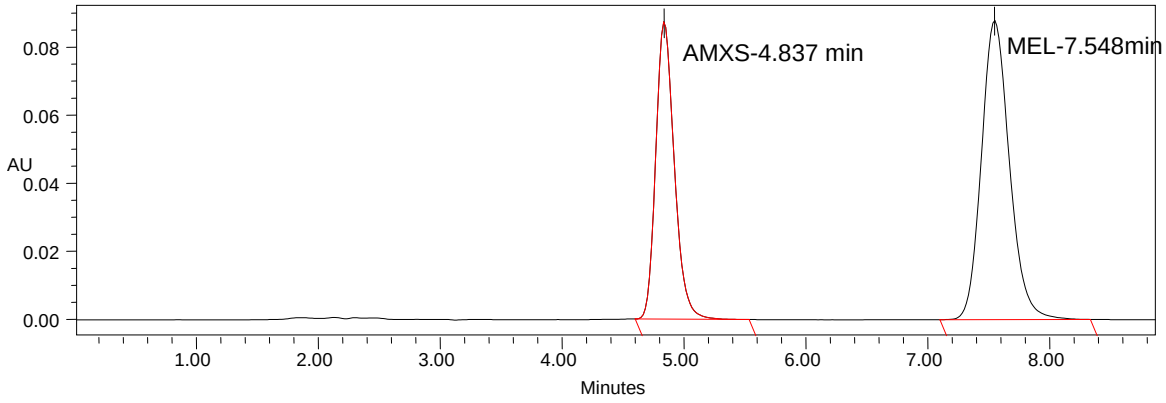


Figure2: Typical HPLC Chromatogram of MEL And AMXS

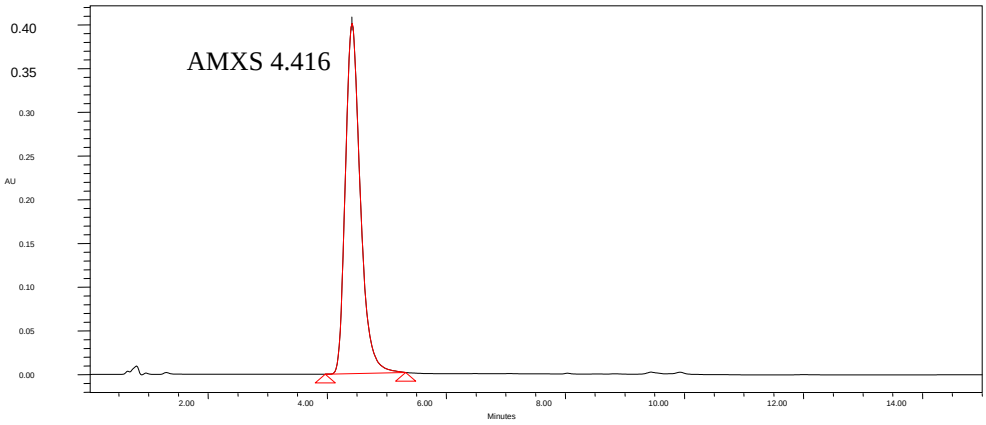


Figure 3: Chromatogram of AMXS API

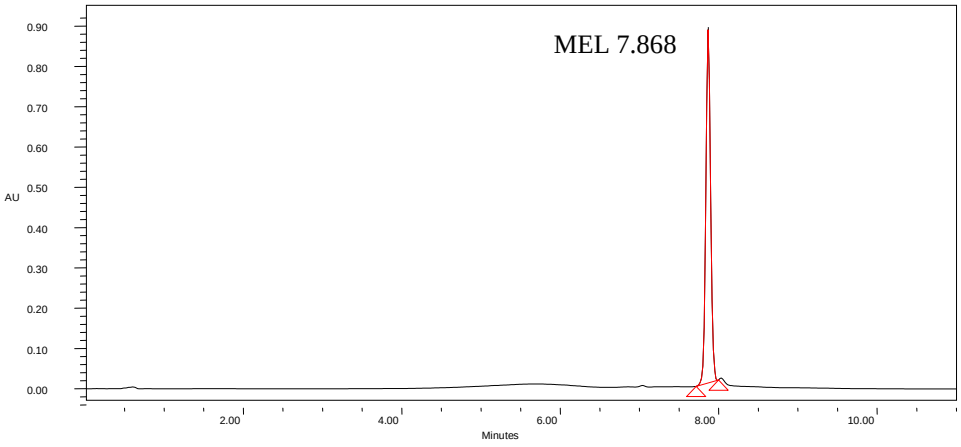


Figure 4: Chromatogram of MEL API

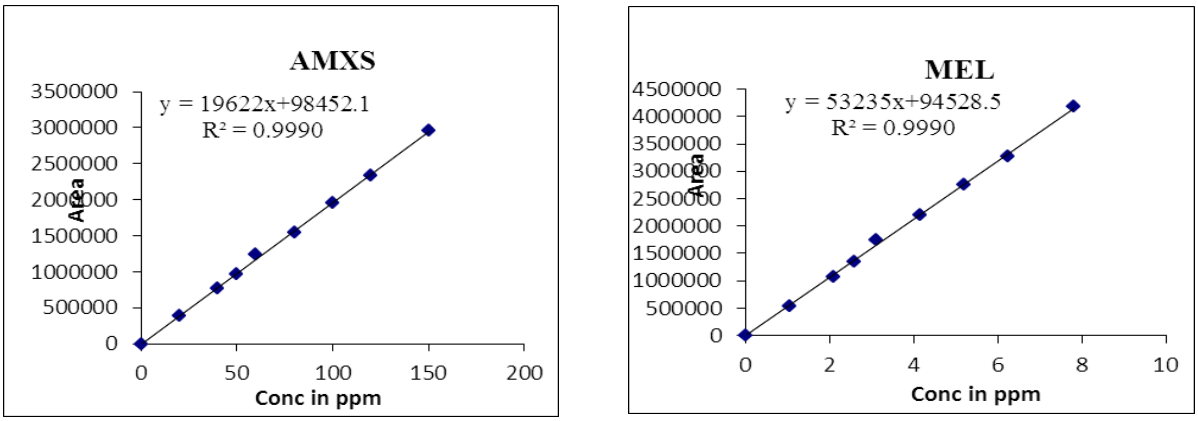


Figure 5: Calibration curves for AMXS and MEL

CONCLUSION

The low standard deviation and %RSD calculated for the proposed developed method and validation were in conformity with standards. Hence, it can be concluded that the developed RP-HPLC method is accurate, precise and selective and can be employed successfully for the simultaneous estimation of MEL and AMXS in injectable dosage form for routine quality control analysis.

ACKNOWLEDGEMENT

The authors are thankful to Nirmala College of Pharmacy Mangalagiri, Guntur, Andhra Pradesh, India for providing necessary facilities to carry out the research work.

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